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TITLE: Phase II Study of a HER-2/neu (HER2) Intracellular Domain (ICD) Peptide-Based Vaccine Administered to Stage IIIB and IV HER2 Positive Breast Cancer Patients Receiving Trastuzumab Monotherapy

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14. ABSTRACT
The primary purpose of this grant is to determine the overall survival (OS) benefit in Stage IIIB and IV HER2 positive breast cancer patients vaccinated with a HER2 intracellular domain (ICD) peptide-based vaccine while receiving maintenance trastuzumab. Patients enrolled will be HER2 overexpressing stage IIIB and IV breast cancer patients who have been treated to a clinical complete response remission or have stable bone only disease and are within 6 months of starting maintenance trastuzumab. The scope of the work includes a Phase II, non-randomized, single arm study of a HER2 ICD peptide-based vaccine given concurrently with trastuzumab. Ten subjects have been enrolled during the last reporting period. All adverse events reported for these ten subjects are grades 1 and 2. There have been no major findings to date.

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INTRODUCTION

The subject of this grant is to determine whether a HER-2/neu (HER2) intracellular domain (ICD) peptide vaccine, administered in combination with trastuzumab, will impact outcomes in patients with Stage IIIB and IV HER2-positive breast cancer. The purpose of this trial is to determine the overall survival benefit in Stage IIIB and IV HER2 positive breast cancer patients vaccinated with a HER2 ICD peptide-based vaccine while receiving maintenance trastuzumab.

The scope of the work includes a Phase II single arm study of a HER2 ICD peptide based vaccine given concurrently with trastuzumab. Patients enrolled will be HER2 overexpressing stage IIIB and IV breast cancer patients who have been treated to a clinical complete remission or have stable bone only disease and are within 6 months of starting maintenance trastuzumab. The primary objective is an estimate of overall survival (OS) compared to a historical control of patients treated with chemotherapy and trastuzumab (55% at 2 years). We hypothesize that the overall survival rate at 2 years with vaccination, if successful, would be 75%. Fifty-two patients will provide 92% power to detect a statistically significant increased survival rate compared to the fixed historical rate of 55% at the one-sided significance level of p=0.05.

Secondary objectives include the assessment of the toxicity of the combined approach as well as the immunogenicity of HER2 ICD peptide vaccination. If there is evidence to suggest that the true rate of Grade IV toxicity exceeds 5% or the true rate of Grade III-IV toxicity exceeds 10% then the trial will be stopped for safety concerns. Immunogenicity of the approach will be evaluated as the ability of the vaccine to elicit HER2 ICD specific T cell immunity, to elicit epitope spreading, and to stimulate both a CD4+ and CD8+ immune response. Immune response and epitope spreading will then be modeled as time-dependent covariates in Cox proportional hazards regression models for OS to assess the correlation of each of these outcomes with the hazard of mortality.

BODY

Task 1*: To assess the potential clinical impact of the administration of a HER2 ICD peptide-based vaccine to Stage IV breast cancer patients receiving concurrent trastuzumab monotherapy*

a. Construct and vial the HER2 ICD peptide vaccine. This task has been completed. The vaccine product (lot 6002) continues to be monitored at specific intervals for product stability. A Stability Study Log for lot 6002 is maintained. The study log lists the testing dates and provides a summary table to record data for each time point tested. All reserved stability vials are stored under the same conditions as the final product, -20 ± 2°C. At each stability time point reserved vials are removed from storage and visually inspected for appearance. MALDI-TOF mass spectrometry and High Performance Liquid Chromatography (HPLC) are used to confirm the stability.

b. Enroll and treat patients. This study was officially approved by the US Army Medical Research and Materiel Command (USAMRMC) Human Subjects Research Review Board (HSRRB) on June 1, 2006.

During the last reporting period we enrolled ten subjects (started December 2006 - May 2008). Table 1 demonstrates the study status of enrolled subjects through May 19, 2008.

Table 1. Study Enrollment Table

Study Time Point	Number of subjects completed to specified time point	Off Study
Vaccine 1	1	0
Vaccine 2	0	0
Vaccine 3	0	0
Vaccine 4	0	0
Vaccine 5	1	1*
Vaccine 6	0	0
FU 1 (Month 7)	1	0
FU 2 (Month 5)	4	0
FU 3 (Month 14)	3	0
FU 4 (Month 18)	0	0
Total	**10**	**1**

*MUGA scan performed by the subject's oncology showed an ejection fraction decrease; subject also developed pneumonia and it was agreed the subject would not return to Seattle for 6th vaccine.

To be eligible for this study, subjects must be enrolled within 6 months of initiating maintenance trastuzumab (Herceptin). As part of the last annual report submitted on May 25, 2007 we were seeking approval from both the Fred Hutchinson

Cancer Research Center – Cancer Consortium IRB and U.S. Army Medical Research and Material Command (USAMRMC) Office of Research Protections (ORP) for the inclusion of Stage IIIB patients on study. At the time of the last reporting period we had obtained FDA approval in December 2006. The IRB approval was granted May 23, 2007 and USAMRMC ORP approved the changes on October 22, 2007.

During the last reporting period we screened 89 breast cancer subjects with 15 being potentially eligible for this study. As of March 10, 2008 we were contacted by Ms. Meg Young of the Congressionally Directed Medical Research Programs (CDMRP) collecting information about the challenges we face in meeting accrual goals for this study. During our conversations we have discussed some of the challenges to accrual with this study as well as some possible suggestions for improvement. Some of these challenges include:

- The ability to identify potential subjects as they are just starting trastuzumab so we can be sure to have all related eligibility documentation collected by the time they begin their maintenance trastuzumab. It is vitally important to this study that we have eligibility criteria approved before they begin their maintenance trastuzumab therapy.
- We have a local cancer center, Seattle Cancer Care Alliance (SCCA), who treats breast cancer patients. However, we have found that a large number of the breast cancer patients coming to the SCCA are seeking a second opinion which means they have already been receiving treatment and usually fall outside of the required window of being 6 months out from the start of maintenance trastuzumab.
- When this study was originally submitted to the DOD (2004) only Stage IV breast cancer patients were being given trastuzumab. Once this study was approved (2006) there were new changes in how trastuzumab was given. The change in standard treatment was due to data from three large, prospective clinical trials (National Cancer Institute trials (NSABP: B-31 and NCCTG: N9831) and Breast International Group (HERA Trial)) reported at the American Society of Clinical Oncology (ASCO) 2005 meeting which demonstrated that trastuzumab clearly improves clinical outcomes for women with HER2 positive, early-stage breast cancer. Collectively, data from these trials showed that those patients with early-stage breast cancer who received trastuzumab with chemotherapy had a significantly improved disease-free survival and overall survival compared to those patients who received chemotherapy alone. Recognizing that this window of treatment is arbitrary although reasonable, many clinicians are also offering trastuzumab therapy to those patients who finished chemotherapy without trastuzumab within the past 12-18 months.

We have taken several steps to improve our enrollment for this study by working closely with groups that specialize in breast oncology. These efforts include:

- An ongoing research collaboration with Breastlink Medical Group, Inc., a large private oncology practice in Southern California, that exclusively treats breast cancer. Breastlink research coordinators regularly review their patient lists for Stage IIIB and IV HER2 positive patients that are beginning trastuzumab therapy. Subjects interested in participating are provided with our Screening Coordinator's contact information. Since January 2005 about two-thirds of the subjects enrolling in our group's studies have been referred by Breastlink. Three of the 10 subjects currently enrolled in this study have been referred by them and an additional 3 potential subjects currently being screened for study. For the three potential subjects we are simply waiting for them to complete their chemotherapy or radiation therapy before they are on maintenance trastuzumab.

  In an effort to promote and encourage routine review of subject records specifically, we have worked closely with Breastlink staff to set up a system of reviewing their patient records, on a regular basis, to keep a consistent pool of potential subjects for this study. This involves reviewing charts for HER2 status, current treatment regimen and their trastuzumab status. This system has been in place about 1 month.

- A collaboration with the Seattle Cancer Care Alliance (SCCA) to review patient records for potential trial eligibility at initial consultation visits. All physicians in the SCCA Breast Cancer Group are aware of the trial inclusion and exclusion criteria and on-site research coordinators provide study information to patients with a potential interest in study participation.

  In addition, we are receiving a weekly list of subjects who are diagnosed as having HER2 positive breast cancer. With this list we are able to look these subjects up in the system, for which they have consented be included, to determine if they are potentially eligible for the study. We then notify the potential subject's physician that their patient is potentially eligible to participate and then we contact the subject to determine their interest.

- We have also continued our conversations with Ms. Young as well as Dr. Katherine Moore (Grants Manager, CDMRP) in how to further modify the protocol to increase accrual, by expanding our patient population, but without significantly altering study design. On April 8, 2008 we had a conference call with Ms. Young and Dr. Moore to discuss our proposed protocol changes as to whether they were appropriate and still allow us to meet our primary endpoint which is clinical outcome. The following items were discussed:
  - Realizing that our original targeted study population (Stage IV patients who are trastuzumab naïve) is rapidly shrinking due to recent changes in standard treatment as discussed above, we discussed the possibility of including Stage IIIC subjects in addition to the Stage IIIB and IV subjects for which we are approved for. This is based on current literature which is showing that Stage IIIC and Stage IIIB patients are similar in terms of treatment (both groups receive both neoadjuvant and adjuvant chemotherapy in combination with trastuzumab for up to 12 months) and RFS and OS is similar in both groups. While interim results from recent phase III trials in HER2 positive patients have demonstrated improved RFS and possibly OS in early stage breast cancer (stage II-IIIA) with the use of trastuzumab in combination with chemotherapy in the adjuvant setting [3]; this has not been shown in patients with stage IIIB or IIIC HER2 positive disease. In fact, recent data from a large retrospective analysis demonstrated that despite advances in the multimodality treatment of stage IIIB breast cancer which includes neoadjuvant chemotherapy, OS has not changed [4]. At best, the 5 year is between 41-47% for patients with HER2 positive stage IIIB and IIIC disease and 5 year RFS is between 33-37%. Thus, this is a population of patients who could possibly benefit from HER2 vaccination. Moreover, this is also a larger population of patients that realistically could be targeted for enrollment. Per Dr. Moore's recommendations we consulted with our statistician, Dr. Ted Gooley regarding changing our primary endpoint to evaluate RFS and OS in Stage IIIB and IIIC as one group collectively. However, at the same time we wanted to keep the sample size (n=52) the same as well as still be able to perform an interim analysis after 25 patients to determine if we should continue enrollment. Equally important was the ability to allow for continued enrollment of Stage IV patients given the limited treatment options for these patients plus we had already enrolled 5 Stage IV patients. Per Dr. Gooley's recommendations the statistical changes described below are being proposed.

Proposed Changes to Statistical Considerations

A. Study statistics

The primary objective of this Phase II trial is to examine the potential efficacy of the proposed treatment in terms of relapse-free survival at 4 years following the start of vaccine. Stopping rules for excess toxicity will also be put in place, and we shall also examine the correlations between immunological response and survival and the presence of epitope spreading and survival.

1. Relapse-free survival assessment. Historical data suggest five-year (after diagnosis) RFS rates for Stage IIIB patients of roughly 38% and for Stage IIIC patients 33-37%. Patients on this trial will be given vaccine approximately one year after diagnosis, and the RFS rate at one year in the Stage IIIB/C patients is roughly 75-80%. The benchmark RFS that we shall use for this trial, therefore, will be 35%/80%= 44% RFS at 4 years following start of vaccination.

If we assume that the true overall survival rate at 4 years after the start of the proposed treatment is 65%, then 52 patients will provide 92% power to detect a statistically significantly increased survival rate compared to the fixed historical rate of 44% at the one-sided significance level of .05. This assumes that 4-year survival is modeled as a binary outcome. After 25 patients have been enrolled with a minimum follow-up of 2 years, an estimate of the RFS at 4 years will be calculated. If one treats the outcome as a binary variable, then 11 or fewer 4-year relapse-free survivors leads to rejection of the alternative hypothesis that 4-year survival is 65% at the significance level of .025. Even though we will be using the Kaplan-Meier (KM) estimate of 4-year survival since possibly not all patients will have complete follow-up to 4 years, we shall translate this result into the requirement for an estimated 4-year RFS of greater than 44% for continuing the trial. In other words, if the KM estimate of RFS at 4 years is 44% or less at the time of analysis (i.e., when the minimum follow-up among the first 25 patients is 2 years), the trial will be stopped for lack of efficacy. If the KM estimate exceeds 44%, then enrollment will be continued to 52 patients. Enrollment will be allowed to continue beyond the 25th enrolled patient until each of the 25 initial patients have completed follow-up to 2 years. The decision to continue enrollment to 52 patients, however, will be based on the outcome of the first 25 patients. Again, treating 4-year survival as a binary outcome, the probability of 30 or more relapse-free survivors to 4 years among 52 patients is .03 if the true 4-year survival is 44%. In other words, we can reject the null

hypothesis that 4-year RFS is 44% at the significance level of .05 if at least 30 of 52 (58%) patients survive to 4 years after start of vaccine. Translating this binary proportion (58%) into a KM estimate of 4-year RFS, we shall consider the proposed treatment to be potentially efficacious if the estimated 4-year RFS is 58% or greater. This estimate will be made when the minimum follow-up among all patients is 2 years.

If this study suggests that there is a RFS benefit (as defined above) associated with the addition of a HER2 ICD vaccine to trastuzumab monotherapy after optimal treatment for Stage IIIB/IIIC breast cancer, then a Phase III randomized trial will be contemplated; trastuzumab and/or chemotherapy followed by maintenance trastuzumab alone vs. trastuzumab and/or chemotherapy followed by maintenance trastuzumab and the HER2 ICD vaccine.

Given the changing population of patients and the fact that we've already enrolled some Stage IV patients, we propose to continue enrolling Stage IV patients until enrollment of the Stage IIIB/C patients is complete, recognizing that the power to detect a statistically significant difference compared to an historical benchmark will be severely limited due to the relative paucity of Stage IV patients. Nonetheless, an observed outcome that is superior to the historical benchmark will be considered to be encouraging in this population.

We will be scheduling a Conference Call with Dr. Moore and Ms. Young in the next couple of weeks to discuss these changes and to get their counsel on making these changes in a manner that will hopefully expediate approval from the CDMRP. Just as we have done in the past we will continue to include Ms. Young and Dr. Moore in the decision making process as their opinions are crucial to how we move forward with this modification.

As for potential subjects currently undergoing screening we have 4 subjects in the final stages of collecting source documents (Table 2). Two of these subjects are Stage IIIB and two are Stage IV. These were identified by Breastlink Medical Group, Inc. using the recent screening system put in place specifically for this study. Even in a short time period this has been a successful tool as we have been able to identify subjects early enough in their care that we will be able to enroll them to the study immediately at the start of their maintenance trastuzumab therapy if they remain eligible by the end of their treatment regimen.

Table 2. Subjects in the Final Phase of Data Collection

Subject	Initial Contact	Source of Referral	State of Residence	Initiation of maintenance trastuzumab
YB	4/15/2008	Breastlink	CA	May 2008
JK	5/2/2008	Breastlink	CA	July 2008
EN	5/2/2008	Breastlink	CA	July 2008
CH	5/2/2008	Breastlink	CA	July 2008

c. Interim statistical analysis after 25 patients have been followed for 1 year. Not applicable for this reporting period. It is understood that once we have enrolled 25 subjects that have been followed for 1 year we should perform an interim analysis of the data.

d. Final analysis of response. Not applicable for this reporting period.

Task 2*: To evaluate the safety of administering a HER2 ICD peptide-based vaccine to Stage IV breast cancer patients receiving trastuzumab monotherapy.*

a. Evaluate immediate toxicity associated with the vaccine. We use the NCI Common Toxicity Criteria (CTC) for Adverse Events Version 3.0 to grade toxicities. We pay particular attention to local reactions associated with the injection site and systemic reactions to include but not limited to fever, malaise, myalgia, nausea and headache. Table 3 is a comprehensive list of adverse events experienced by all subjects on study.

Table 3. Comprehensive List of Adverse Events Reported

ID	CTC Category	Adverse Event	Grade	Attribution	Comments
12001	Allergy/Immunology	Allergic rhinitis (including sneezing, nasal stuffiness, postnasal drip)	1	2	
12004	Allergy/Immunology	Allergic rhinitis (including sneezing, nasal stuffiness, postnasal drip)	1	2	

ID	CTC Category	Adverse Event	Grade	Attribution	Comments
12007	Allergy/Immunology	Allergic rhinitis (including sneezing, nasal stuffiness, postnasal drip)	1	3	
12008	Allergy/Immunology	Allergic rhinitis (including sneezing, nasal stuffiness, postnasal drip)	1	1	
12004	Auditor/Ear	Auditory/Ear - Other (Specify, __)	1	1	Tender extermal ear Lt canal
12003	Blood/Bone Marrow	Hemoglobin	1	2	Low
12004	Blood/Bone Marrow	Hemoglobin	1	2	Low
12005	Blood/Bone Marrow	Hemoglobin	1	2	Low
12007	Blood/Bone Marrow	Hemoglobin	1	2	
12007	Blood/Bone Marrow	Hemoglobin	1	1	
12009	Blood/Bone Marrow	Hemoglobin	1	1	
12005	Blood/Bone Marrow	Hemoglobin	2	2	
12007	Blood/Bone Marrow	Hemoglobin	2	3	
12001	Blood/Bone Marrow	Leukocytes (total WBC)	1	2	
12003	Blood/Bone Marrow	Leukocytes (total WBC)	1	2	
12004	Blood/Bone Marrow	Leukocytes (total WBC)	1	2	
12006	Blood/Bone Marrow	Leukocytes (total WBC)	1	3	
12006	Blood/Bone Marrow	Leukocytes (total WBC)	1	2	
12008	Blood/Bone Marrow	Leukocytes (total WBC)	1	3	
12008	Blood/Bone Marrow	Leukocytes (total WBC)	1	3	
12008	Blood/Bone Marrow	Leukocytes (total WBC)	1	3	
12009	Blood/Bone Marrow	Leukocytes (total WBC)	1	3	
12009	Blood/Bone Marrow	Leukocytes (total WBC)	1	3	
12003	Blood/Bone Marrow	Leukocytes (total WBC)	2	3	increase from baseline
12001	Blood/Bone Marrow	Lymphopenia	1	3	
12002	Blood/Bone Marrow	Lymphopenia	1	3	
12003	Blood/Bone Marrow	Lymphopenia	1	2	
12006	Blood/Bone Marrow	Lymphopenia	1	3	
12006	Blood/Bone Marrow	Lymphopenia	1	2	
12008	Blood/Bone Marrow	Lymphopenia	1	3	
12008	Blood/Bone Marrow	Lymphopenia	1	3	
12009	Blood/Bone Marrow	Lymphopenia	1	3	
12005	Blood/Bone Marrow	Lymphopenia	2	1	
12006	Blood/Bone Marrow	Lymphopenia	2	2	
12003	Blood/Bone Marrow	Neutrophils/granulocytes (ANC/AGC)	1	2	Neutropenia
12007	Blood/Bone Marrow	Neutrophils/granulocytes (ANC/AGC)	1	3	Neutropenia

ID	CTC Category	Adverse Event	Grade	Attribution	Comments
12009	Constitutional Symptoms	Constitutional Symptoms - Other (Specify, __)	1	2	Shakiness
12003	Constitutional Symptoms	Fatigue (asthenia, lethargy, malaise)	1	1	
12003	Constitutional Symptoms	Fatigue (asthenia, lethargy, malaise)	1	2	
12005	Constitutional Symptoms	Fatigue (asthenia, lethargy, malaise)	1	1	
12006	Constitutional Symptoms	Fatigue (asthenia, lethargy, malaise)	1	5	
12007	Constitutional Symptoms	Fatigue (asthenia, lethargy, malaise)	1	2	Secondary to pushing herself
12008	Constitutional Symptoms	Fatigue (asthenia, lethargy, malaise)	1	1	
12009	Constitutional Symptoms	Fatigue (asthenia, lethargy, malaise)	1	1	
12001	Constitutional Symptoms	Fatigue (asthenia, lethargy, malaise)	2	2	
12003	Constitutional Symptoms	Fatigue (asthenia, lethargy, malaise)	2	1	
12003	Constitutional Symptoms	Fatigue (asthenia, lethargy, malaise)	2	2	
12006	Constitutional Symptoms	Fatigue (asthenia, lethargy, malaise)	2	3	
12007	Constitutional Symptoms	Weight gain	2	1	Fluid retention
12008	Dermatology/Skin	Bruising (in absence of Grade 3 or 4 thrombocytopenia)	1	1	Distal to R great toe
12003	Dermatology/Skin	Dermatology/Skin - Other (Specify, __)	1	3	eczema
12006	Dermatology/Skin	Dermatology/Skin - Other (Specify, __)	1	1	Abrasion, secondary dog claw
12006	Dermatology/Skin	Dermatology/Skin - Other (Specify, __)	1	1	Scalp Redness
12006	Dermatology/Skin	Hair loss/alopecia (scalp or body)	2	1	Secondary to Radiation
12008	Dermatology/Skin	Injection site reaction/extravasation changes	1	5	
12001	Dermatology/Skin	Pruritus/itching	1	3	
12005	Dermatology/Skin	Pruritus/itching	1	3	
12001	Dermatology/Skin	Rash/desquamation	1	3	
12002	Dermatology/Skin	Rash/desquamation	1	2	
12004	Dermatology/Skin	Rash/desquamation	1	1	Heat rash
12005	Dermatology/Skin	Rash/desquamation	1	3	Patch on Left Wrist
12006	Dermatology/Skin	Rash/desquamation	1	1	Facial Rash - Secondary to Radiation
12005	Endocrine	Hot flashes/flushes	1	1	
12003	Gastrointestinal	Anorexia	1	2	
12006	Gastrointestinal	Anorexia	1	2	Secondary to Radiation
12006	Gastrointestinal	Constipation	1	2	
12001	Gastrointestinal	Diarrhea	1	2	
12004	Gastrointestinal	Diarrhea	1	1	

ID	CTC Category	Adverse Event	Grade	Attribution	Comments
12007	Gastrointestinal	Diarrhea	1	1	
12007	Gastrointestinal	Distension/bloating, abdominal	1	1	
12002	Gastrointestinal	Gastrointestinal - Other (Specify, __)	1	2	Coating on Tongue
12003	Gastrointestinal	Gastrointestinal - Other (Specify, __)	1	1	Possible GERD
12007	Gastrointestinal	Gastrointestinal - Other (Specify, __)	1	3	Cramping
12003	Gastrointestinal	Nausea	1	2	
12006	Gastrointestinal	Nausea	1	1	
12007	Gastrointestinal	Nausea	1	3	
12003	Gastrointestinal	Vomiting	1	1	
12003	Gastrointestinal	Vomiting	1	3	1 episode
12003	Gastrointestinal	Vomiting	1	1	
12007	Gastrointestinal	Vomiting	1	1	Secondary to Stressors
12005	Hemorrhage/Bleeding	Hemorrhage/Bleeding - Other (Specify, __)	1	2	Vaginal Spotting
12003	Infection	Infection - Other (Specify, __)	1	1	Port a cath
12006	Infection	Infection - Other (Specify, __)	1	1	Upper Respiratory Infection
12009	Metabolic/Laboratory	Albumin, serum-low (hypoalbuminemia)	1	3	
12009	Metabolic/Laboratory	Alkaline phosphatase	1	1	
12001	Metabolic/Laboratory	AST, SGOT(serum glutamic oxaloacetic transaminase)	1	3	
12006	Metabolic/Laboratory	Calcium, serum-low (hypocalcemia)	1	1	
12003	Metabolic/Laboratory	Potassium, serum-low (hypokalemia)	1	1	
12008	Metabolic/Laboratory	Potassium, serum-low (hypokalemia)	1	2	
12002	Metabolic/Laboratory	Proteinuria	1	3	
12006	Musculoskeletal/Soft Tissue	Musculoskeletal/Soft Tissue - Other (Specify, __)	1	2	Rt. Bony area-clavicle area
12003	Neurology	Cognitive disturbance	1	1	
12006	Neurology	Confusion	1	1	Secondary to Radiation
12005	Neurology	Memory impairment	2	1	Chemo brain
12003	Neurology	Mood alteration	1	1	Anxiety
12006	Neurology	Neurology - Other (Specify, __)	1	2	Nervousness - Secondary to Brain Mets
12006	Neurology	Neurology - Other (Specify, __)	1	1	Depression
12007	Neurology	Neurology - Other (Specify, __)	1	2	Short term memory change

ID	CTC Category	Adverse Event	Grade	Attribution	Comments
12007	Neurology	Neurology - Other (Specify, __)	1	1	Anxiety
12004	Neurology	Neurology - Other (Specify, __)	2	1	Depression, secondary to cancer dx
12004	Neurology	Neurology - Other (Specify, __)	2	1	Anxiety, secondary to cancer dx
12006	Neurology	Neurology - Other (Specify, __)	2	1	New brain mets
12007	Neurology	Neuropathy: sensory	1	3	
12002	Pain	Pain	1	3	Back
12003	Pain	Pain	1	1	Heacache
12006	Pain	Pain	1	2	Head/headache
12007	Pain	Pain	1	3	Head/headache
12001	Pain	Pain - Other (Specify, __)	1	3	Myalgias
12003	Pain	Pain - Other (Specify, __)	1	1	Sore Throat
12004	Pain	Pain - Other (Specify, __)	1	2	Rt Thumb Proximal Joint Pain
12006	Pain	Pain - Other (Specify, __)	1	1	Myalgias
12006	Pain	Pain - Other (Specify, __)	1	1	Left Forearm
12007	Pain	Pain - Other (Specify, __)	1	3	Myalgias/Arthralgias to wrist
12009	Pain	Pain - Other (Specify, __)	1	3	Arthralgias
12009	Pain	Pain - Other (Specify, __)	1	3	Knee
12002	Pulmonary/Upper Respiratory	Cough	1	1	
12008	Pulmonary/Upper Respiratory	Nasal cavity/paranasal sinus reactions	1	2	Dry B Nares
12004	Pulmonary/Upper Respiratory	Pulmonary/Upper Respiratory - Other (Specify, __)	1	2	Rhinitis secondary to Herceptin
12004	Pulmonary/Upper Respiratory	Pulmonary/Upper Respiratory - Other (Specify, __)	1	2	Nasal Congestion
12006	Pulmonary/Upper Respiratory	Pulmonary/Upper Respiratory - Other (Specify, __)	1	1	Pharyngitis
12006	Pulmonary/Upper Respiratory	Pulmonary/Upper Respiratory - Other (Specify, __)	1	1	URI
12007	Pulmonary/Upper Respiratory	Pulmonary/Upper Respiratory - Other (Specify, __)	1	2	Right hilar lung lymphadenopathy recurrence
12008	Pulmonary/Upper Respiratory	Pulmonary/Upper Respiratory - Other (Specify, __)	1	1	URI
12008	Pulmonary/Upper Respiratory	Pulmonary/Upper Respiratory - Other (Specify, __)	1	1	URI
12003	Pulmonary/Upper Respiratory	Pulmonary/Upper Respiratory - Other (Specify, __)	2	1	URI

ID	CTC Category	Adverse Event	Grade	Attribution	Comments
12004	Pulmonary/Upper Respiratory	Pulmonary/Upper Respiratory - Other (Specify, __)	2	1	URI
12005	Pulmonary/Upper Respiratory	Pulmonary/Upper Respiratory - Other (Specify, __)	2	1	URI
12006	Renal/Genitourinary	Cystitis	2	1	
12005	Renal/Genitourinary	Renal/Genitourinary - Other (Specify, __)	1	2	Dysuria
12005	Renal/Genitourinary	Renal/Genitourinary - Other (Specify, __)	1	3	burning with urination
12006	Renal/Genitourinary	Renal/Genitourinary - Other (Specify, __)	1	1	WBC's in Urine
12008	Renal/Genitourinary	Renal/Genitourinary - Other (Specify, __)	1	3	Trace glucose in urine
12009	Renal/Genitourinary	Renal/Genitourinary - Other (Specify, __)	1	2	1+ WBC
12009	Renal/Genitourinary	Renal/Genitourinary - Other (Specify, __)	1	1	1+ WBC and RBC (Asymptomatic)
12005	Renal/Genitourinary	Renal/Genitourinary - Other (Specify, __)	2	2	UTI
12005	Renal/Genitourinary	Urine color change	1	1	Blood in Urine - Secondary to vag. Bleeding
12007	Syndromes	Flu-like syndrome	1	1	
12009	Syndromes	Flu-like syndrome	1	1	

External Monitoring

As part of our Data Safety Monitoring Plan an independent monitor, assigned by the Clinical Trials Support Office at the Fred Hutchinson Cancer Research Center (FHCRC), verifies consent documentation for all newly enrolled subjects in addition to reviewing data collected since the previous monitoring visit for randomly selected subjects. All regulatory documentation is reviewed including all IND documentation.

We were monitored twice since the last reporting period: August 29-30, 2007 and February 5-6, 2008. There were no major findings.

We are due again for monitoring in August 2008.

Medical Monitor Review

According to our Data Safety Monitoring Plan (DSMP) we are scheduled to meet with the Medical Monitor and related clinical research staff members quarterly. Prior to each meeting the Medical Monitor, Dr. Disis and related clinical research staff members will be provided with an agenda, a Safety and Performance Report which includes total enrollment, adverse event reporting for and recently approved modifications and amendments for the reporting period.

All meeting minutes are reviewed, approved and signed by the Medical Monitor before submitting the information to the Fred Hutchinson Cancer Research Center – Cancer Consortium IRB for review.

Since the last Annual Report we have met with our Medical Monitor on June 22, 2007, December 27, 2007 and March 17, 2008. It should be noted that in the last Annual Report (Amendment 0.0041) we reported these meetings were to be conducted bi-annually (twice a year). After a recent Monitoring Visit by the Fred Hutchinson Cancer Research Center – Clinical Trials Support Office, in February 2008, they documented that the DSMP stated these meetings were to occur quarterly and not bi-annually. We are now on track, but we are reporting that the 2007 DSMP meetings were spaced out by 6 months.

In the next modification to the protocol we will be changing the DSMP meetings with the Medical Monitor to bi-annually (twice-a-year) rather than quarterly as this is a phase II study and our adverse events are mostly grades 1 and 2.

Our next Data Safety Monitoring Plan meeting is scheduled for June 2008.

b. Determine whether there is any cardiac toxicity associated with the co-administration of the HER2 ICD peptide based vaccine with trastuzumab. When subjects are enrolled we will closely monitor and document any abnormal cardiac events observed by us at clinic visits or reported to us by the subjects or physicians. All subjects have documentation of a MUGA scan within 6 months for eligibility assessment and if that MUGA scan is greater than 60 days old at time of enrollment we perform a MUGA scan at their baseline visit. A follow-up MUGA scan is performed again at 4 months post-vaccine. Table 4 compares ejection fractions at baseline and 4 Months Post-Last Vaccination.

Table 4: Baseline and 4 Month Post-Last Vaccine EF Evaluation

Subject # (n=9)	Pre-vaccine EF	4 months post-vaccine EF
12001	68%	60-65% (Echocardiogram)
12002	61%	65%
12003	65%	52%
12004	64%	Have not yet received
12005	59%	57.5%
12006	64%	61%
12007	60%	Have not yet received
12008	66%	51-53%
12009	56%	45.8% [a]

[a] Primary oncologist is aware of EF drop. The subject's oncologist has scheduled a repeat MUGA for the of May 2008. We will obtain a copy of this documentation as soon as it is available.

As part of our recent modification approved by USAMRMC ORP on October 22, 2008, we requested that an echocardiogram as well as a MUGA scan be allowed as a means of monitoring cardiac function for the study. However, the one condition of this is that the results must be comparable between baseline and follow-up; for example if a subject's baseline test is a MUGA then the repeat test must also be a MUGA. The same goes for the echocardiogram. Otherwise it is a deviation from protocol.

c. Evaluate for any potential toxicities due to the generation of an immune response to HER2. The toxicities we would expect to see for an autoimmune response to HER2 would include: (1) skin reactions such as rashes, (2) gastrointestinal events such as severe diarrhea, (3) pulmonary events, (4) change in kidney function such as a change in creatinine or (5) cardiotoxicity.

All of these toxicities are closely monitored, by a credentialed clinician such as a physician and/or physician's assistance, at each clinic visit. These toxicities are recorded and monitored by routine review of systems, clinical laboratory results, and other clinical assessment (i.e. chest x-rays, MUGAs, etc.).

To date our toxicity reporting does not indicate any of our ten subjects have developed an immune response to HER2 has developed. All toxicities on study have been of a low grade either grades 1 or 2 (Table 3). In addition, we have not observed or had reported to us by a patient or their physician any symptoms indicating an immune response to HER2 has occurred in any of our subjects. At this time we are confident we are not generated an immune response to HER2.

Task 3*: To determine the immunogenicity of a HER2 ICD peptide-based vaccine in patients with Stage IV breast cancer receiving concurrent trastuzumab monotherapy*

a. Determine the immunogenicity of the approach by assessing the T cell response to HER2 ICD. We have evaluated the T cell responses to the three ICD peptides included in this vaccine and overlapping peptide pools for the HER2 intracellular domain (ICDpm) using a standard 10 day IFN-gamma(g) ELISpot assay in eight patients. In this assay, PBMN before and after vaccinations were stimulated with p776, p927, p1166 and ICDpm respectively on Day 1, and re-stimulated on Day 8. The spots of IFN-g secreted after the stimulations were counted on day 10 using an ELISpot plate reader. Our results show that p776 specific response (antigen specific cells/10^6 PBMN) increased 5 fold (pre vs. post: 74 ± 42 vs. 374 ± 143; mean ± SE; n=8. p=0.063), the p927 specific response increased 12 fold (pre vs. post: 39 ± 23 vs. 465 ± 184; p=0.037), and p1166 response increased 6 fold (pre vs. post 101 ± 48 vs. 618 ± 201; p=0.025) after the vaccination. The response to ICDpm increased 5 fold (pre vs. post: 126 ± 56 vs. 599 ± 198; p=0.037). Our group has previously established that ICDpm are equivalent to HER2 recombinant protein. Thus, the response to ICDpm may be an indicator of successful immunization. Among the eight patients, seven (88%) developed immunity to HER2 ICD peptides. One of the

patients had higher pre-existent immunity to the HER2 peptides; her response did not further increase. In contrast, the response to tetanus toxoid (TT) did not increase post vaccination (p=0.512). Figure 1 shows these results.

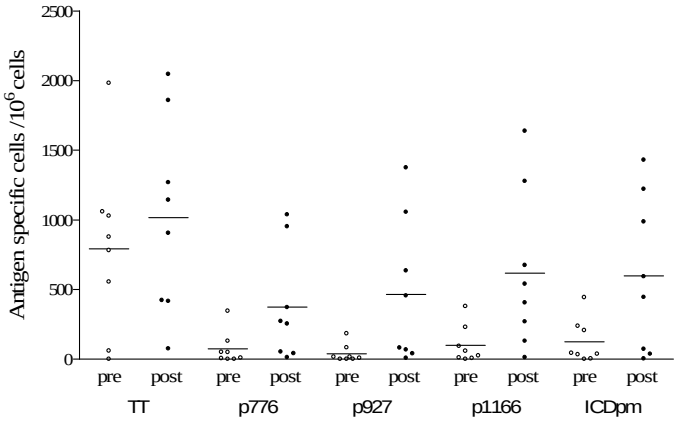

Figure 1. HER2 ICD peptide and protein T cell immunity elicited after the active vaccination. The bars indicate the mean.

b. Determine the incidence of epitope spreading to the HER2 ICD or other peptides in the immunizing mix (intermolecular epitope spreading). We have evaluated the T cell responses to overlapping peptide pools for the HER2 extracellular domain (ECD pm), which is not included in the vaccine. We found that patients developed significant responses to ECDpm (pre vs. post: 75 ± 31 vs. 471 ± 181; n=8; p=0.049) (Figure 2). Among the eight patients, five (63%) developed epitope spreading. Our group has recently demonstrated that the patient's survival was significantly associated with the development of epitope spreading following vaccination.

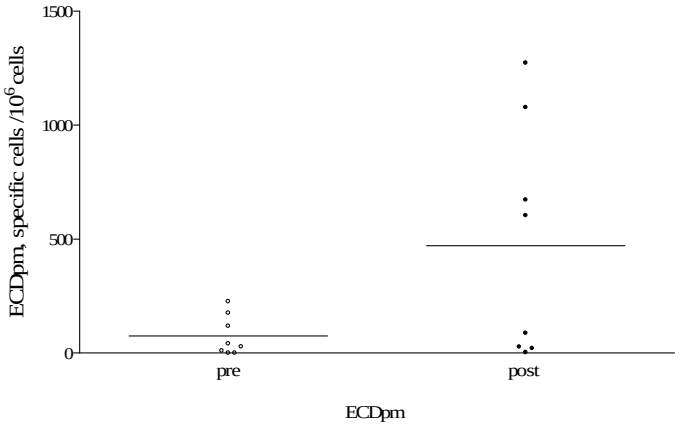

Figure 2. HER2 ECD immunity elicited after ICD peptide vaccination. The bars indicate the mean.

In addition, we evaluated the serum levels of TGF-beta (b) in patients before and after vaccination using a human TGFb1 ELISA kit (eBioscience, San Diego, CA). TGF-b is an immunosuppressive cytokine secreted by tumor and Treg cells. We found that the levels of serum TGFb decreased in 7 of the 9 patients evaluated after vaccination. The mean level of hTGFb was 2,269 (± 857) pg/ml before the vaccination, and decreased to 1276 (± 381) pg/ml after 3rd vaccine and maintained at 1293 (± 609) pg/ml after 6th vaccine (mean ± SE, n=9; Figure 3). Thus, the mean level of serum TGFb decreased more than 40% after vaccination, although it did not reach significant differences. Our group has demonstrated that serum levels of TGFb is correlated with levels of $CD4^+CD25^+$ Treg cells. The decreased levels of serum TGFb may be an indicator that the Treg were not increased during the CD4+ targeted immunization, and may predict a better prognosis as elevated levels of serum TGFb are associated with an increased risk of relapse in breast cancer patients (Bates GJ et al J Clin Oncol 2006). We will further evaluate the levels of TGFb in more patients who are enrolled in this protocol.

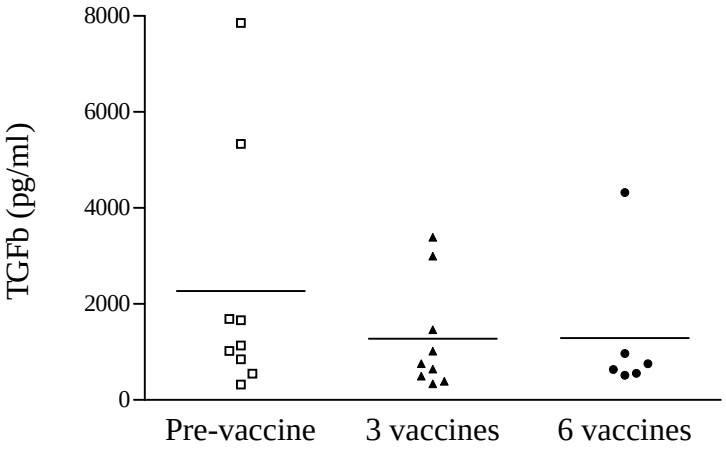

Figure 3. Levels of TGFb decreased in the serum of patients after vaccination with HER2 ICD peptides. The bars indicate the mean.

c. Determine the incidence of epitope spreading to other immunogenic proteins associated with breast cancers (extramolecular epitope spreading). Not applicable to this reporting period.

d. Assess the absolute magnitude of the CD4+ and CD8+ HER2 specific immune responses generated after active immunization. Not applicable to this reporting period.

e. Evaluate the generation of HER2 specific antibody immunity and antibody avidity. Not applicable to this reporting period.

f. Determine whether overall survival is associated with the development of HER2 specific T cell response or epitope spreading after active immunization. Not applicable to this reporting period.

KEY RESEARCH ACCOMPLISHMENTS

Not applicable to this reporting period.

REPORTABLE OUTCOMES

Included in this annual report is an abstract submitted for the Era of Hope Meeting entitled: "Phase II study of a HER-2/neu Peptide-Based Vaccine plus Concurrent Trastuzumab for Prevention of Breast Cancer Relapse".

CONCLUSIONS

We began study enrollment on December 29, 2006. We have since enrolled ten subjects who are at varying phases of vaccination. To date we have observed only low grade adverse events (Grades 1 & 2) most of which were expected and unrelated to the vaccine.

We are aggressively pursuing strategies to increase our accrual to this study by working with our established research and clinical collaborators both locally as well as throughout the country, particularly the west coast. Since the last reporting period we have obtained approval to include Stage IIIB subjects to be enrolled in this study along with the originally approved Stage IV breast cancer patients. We have also been working closely with members of CDMRP in an effort further broaden our study population to include Stage IIIC subjects. We will continue to work closely with CDMRP, specifically Ms. Young and Dr. Moore, to submit the modification to include Stage IIIC subjects. Once the modification is approved at our IRB or record, we will forward these modifications to the USAMRMC HSRRB and FDA for approval prior to implementation.

In order to successfully accomplish the scope of work for this project, we anticipate the need for a 12 month no-cost extension to allow time to enroll and treat the remaining patients and complete data analysis. This request has already been submitted to Dr. Moore the Grant Manager.

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